May 4th, 12:00 AM

The Involvement of Ubiquitin in Med13 Cyclin C Degradation Following Cellular Stress

Ayesha Gurnani
Rowan University

Brittany Friedson
Rowan University

Katrina Cooper
Rowan University

ROWAN-VIRTUA
School of
Osteopathic Medicine

The Involvement of Ubiquitin in Med13/Cyclin C Degradation Following Cellular Stress

Ayesha Gurnani OMS-II, Brittany Friedson PhDc, Katrina Cooper PhD
Department of Molecular Biology

INTRODUCTION

The Cdk8 Kinase Module is a dissociable regulator of cellular stress response genes, with degradation of its components Med13 and cyclin C eventually determining cell fate decisions such as engaging cell survival or cell death mechanisms following exposure to environmental stress (Figure 1). Nitrogen starvation is a stressor known to induce autophagy for cell survival. Our lab recently found that Med13 and cyclin C of the Cdk8 Kinase Module require ubiquitin for their degradation following this stress[5], including the E2 ubiquitin-conjugating enzymes Ubc4 and Ubc5. Here, we aimed to uncover the roles of ubiquitin with degradation of the Cdk8 Kinase Module following nitrogen starvation, and asked the following questions:

1. Is Doa4 required for the autophagic degradation of Med13?

2. Does Med13 degradation require K63 ubiquitin linkage?

3. What role does ubiquitin play in Med13 degradation?

METHODS

Western blot analysis was conducted to observe the nitrogen starvation-induced degradation of Med13-HA in wild-type, *doa4Δ*, and K63R yeast strains; degradation of cyclin C-MYC in wild-type and K63R strains; and Atg8-GFP activity in wild-type and *ubc4/5Δ* strains. Pgk1-GFP was used as a loading control.

RESULTS

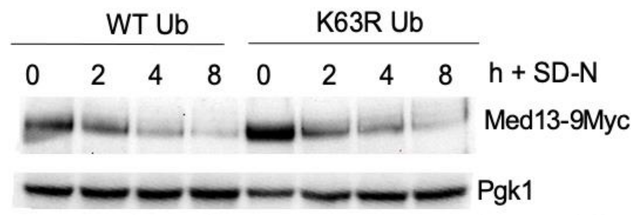

***Figure 2**. Yeast subjected to nitrogen starvation show no difference in Med13 degradation kinetics in wild-type strains as compared to K63R strains.*

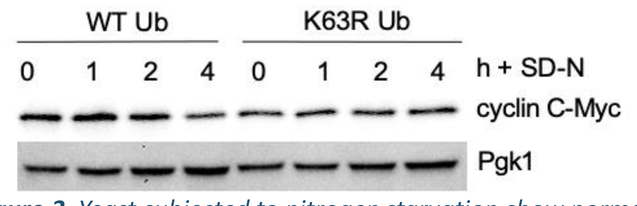

***Figure 3**. Yeast subjected to nitrogen starvation show normal degradation of cyclin C in wild-type strains and stabilization of cyclin C in K63R strains.*

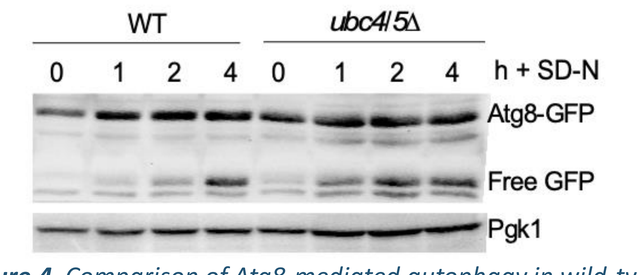

***Figure 4**. Comparison of Atg8-mediated autophagy in wild-type strains and ubc4/5Δ strains shows that Atg8-GFP still gets cleaved in the mutant strain.*

The Cdk8 Kinase Module

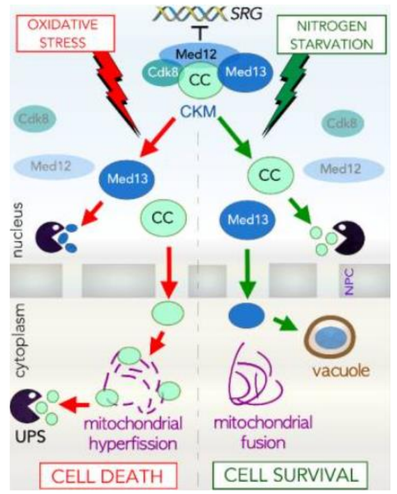

***Figure 1**. Dissociation of the Cdk8 Kinase Module in response to cellular stress, adapted from Friedson et al.[5]*

CONCLUSIONS

1. Doa4 does not have a role in Med13 degradation following nitrogen starvation (not pictured in Results).

2. Med13 degradation does not require K63 ubiquitin linkage (Figure 2); however, cyclin C does appear to require K63 linkage for degradation (Figure 3).

3. Data suggest that the role of Ubc4/5 in Med13 autophagy is specific (Figure 4).

REFERENCES

Special thanks to the members of the Cooper and Strich laboratories at Rowan-Virtua SOM for teaching and guiding me.